Evaluation of IgG Avidity for Cytomegalovirus Infection among Pregnant Women, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

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A Dissertation

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Date: / /2018
Evaluation of IgG Avidity for Cytomegalovirus Infection among Pregnant Women, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

Date: 17/ 4/2018
Declaration

Iam Abeer Ajeib Ibrahim Mohamed, I hereby declare the work that concern the requirement of M.Sc. degree in the Evaluation of IgG Avidity for Cytomegalovirus Infection among Pregnant Women, Wad Medani Maternity Teaching Hospital, Gezira State. This work has been done under the supervision of Dr. Albadawi Abdalbagi Talha, by my own potential and it had not been copied from any other source, also it had not been presented by any other researcher for scientific degree elsewhere.

Name: Abeer Ajeib Ibrahim Mohamed
Place: Wad Medani
Date: March 2018
Dedication

To who are meaning to me all life from beginning childhood until now, support me and stand with me step by step; my father and my mother. To my partner in this life my other half, my husband, who encouraged me. To my children the piece of my heart Ahmed, Aseel and Rateel. To my brothers and sisters

Friends and teachers

To all of them I dedicate this work.
Acknowledgements

Firstly, I thank god, for success in reaching this educational stage and help me to complete this work. This thesis is been possible and completed as result of the contribution and supportance from several great people; who encouraged me and guidanced me in the preparation and completion of this work, therefore, it is with immense gratitude that I acknowledge the support and help of those individuals.

I would like to thank my supervisor Dr. Albadawi Abdelbagi Talha for his unlimited support and help, valuable guidance and comments during the study. Special thank for my friends and great thanks to the Department of Microbiology, Faculty of Medical Laboratory Sciences University of Gezira. Special thanks goes to the Obstetrics and Gynecology Teaching Hospital, Wad Medani, Gezira State, and for Zain Referance Lab; the lab where the practical has been done. Special thank to Ustaz Yousif Abdalhameed for helping me in the analysis of results, To Ustaz Mohamed Yousif who helped me in finishing and organizing this thesis.

Finally, grateful thanks to those whom their samples taken for the current study and I wish them all the best.
Evaluation of IgG Avidity for Cytomegalovirus Infection among Pregnant Women, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

Abeer Ajeib Ibrahim Mohamed

Abstract
Avidity is defined as the aggregate strength with which a mixture of polyclonal IgG molecules binds to multiple antigenic epitopes of proteins. It gradually matures over several months reflective of antigen-driven selection of B cells producing IgG of increasing affinity. IgG antibodies produced during the first few months following primary infection exhibit low avidity whereas antibodies produced by 6 months post infection exhibit high avidity. This descriptive cross sectional study was aimed to evaluate the IgG avidity of human cytomegalovirus among the pregnant women’s attending the outpatient clinic, Obstetrics and Gynecology Teaching Hospital, Wad Medani, Gezira State during the period between October 2017 to December 2017. About 3ml of blood samples were taken from 46 pregnant febrile women their age ranged from 18 to more than 35 years old. Data were collected using questionnaire and were analyzed by using SPSS. Specimens were analyzed used enzyme linked immunosorbent assay utilizing urea as the dissociating agent. Results were expressed as a relative avidity index (RAI). The results showed that there were no correlation between Urban and Rural area, 91.3% within high avidity and 8.7% within equivocal range for both. There were significant correlation between IgG avidity and age group (18 – 26) years have 100% high avidity, other age groups (27 – 34) years 80% high avidity, 20% equivocal range, and more than 35years, were 90% high avidity,10% equivocal range. There were no correlation between IgG avidity and trimester (first trimester 90% high avidity ,10% equivocal range, second trimester 89.4% high avidity,10.6% equivocal range, third trimester 94.1% high avidity, 5.9% equivocal range). There were significant correlation between IgG avidity and house wives 89.7% high avidity, 10.3% equivocal range. The study recommended that the IgG avidity test for Cytomegalovirus should done with each IgG assays during pregnancy.
تقييم طغيان القلب المائي (ج) على عدوى الفيروس المضخم للخلايا بين النساء الحوامل، مستشفى ود مدني التعليمي للأمهات، ولاية الجزيرة، السودان (2017)
عبير عجيب إبراهيم محمد
ملخص الدراسة

يتم تعريف الطمان على أنه القوة الكلية التي يرتبط بها خليط من جزيئات القلوبيين المناعي ج متعددة النسيلة مع حواتم بروتينية مستضدية متعددة من البروتينات. وينضح تدريجياً على مدى عدة أشهر ويعكس اختيار الخلايا البائية التي تعتمد على المستضد لإنتاج القلوبيين المناعي ج لزيادة التقارب. تظهر الأجسام المضادة القلوبيين المناعي ج التي تم إنتاجها خلال الأشهر القليلة الأولى بعد العدوى الأولية انخفاض طفيف في حين أن الأجسام المضادة التي تنتجها 6 أشهر بعد العدوى تظهر تحراً عالية. استهدفت هذه الدراسة الوصفية المقطوعة تقييماً لطائفة القلوبيين المناعي ج من الفيروس المضخم للخلايا البشرية بين النساء الحوامل اللواتي يحضرن الرعاية الخارجية لمجتمع التوليد وأمراض النساء، ود مدني، ولاية الجزيرة خلال الفترة بين أكتوبر 2017 وديسمبر 2017. حوالى 3 مل من عينات الدم أخذت من 46 امرأة حميمة حامل تتراوح أعمارهم بين 18 إلى أكثر من 35 سنة. تم جمع البيانات باستخدام الاستبيان ومثل تحليلها بمثابة الخصائص العامة للعلوم الاجتماعية. وقد تتم تحليل البيانات المستخدمة اختيار المناعة المتصلة المرتبطة الانزيم استخدام اليوريا كعامل الانفصالية. تم التعبير عن النتائج على أنها مؤشر طموح نسبي (RAI).

أوضح النتائج عدم وجود ارتباط بين المناطق الحضرية والريفية، حيث بلغت نسبة 91.3٪ في حالة الطمانينة العالية و 8.7٪ ضمن نطاق الحدود لكلهما. كانت هناك علاقة ملحوظة بين الطفاح القلوبيين المناعي ج والالفونا العميقة (18 - 26) عانا لديهم طموح عالي بنسبة 100٪، والمجموعات العميقة الأخرى (27 - 34) سنة، وكان معدل الطفاح بنسبة 80٪، و 20٪ من مدى التباين، وأكثر من 35 عاناً أعلى بنسبة 90٪ الطمانينة، نسبة نطاق الحدود 10٪. لم تكن هناك علاقة بين طفاح القلوبيين المناعي ج والثاني (الربع الأول من الحمل بنسبة 90٪ عالياً، نسبة نطاق الحدود 10٪، الربع الثاني نسبة إرتفاع 89.4٪، نسبة نطاق الحدود 10٪، الربع الثالث 59.4٪ نسبة عالياً، 5.9٪ مدى الحدود). كانت هناك علاقة ملحوظة بين طفاح القلوبيين المناعي ج وربات المنزل بنسبة عالية بلغت 89.7٪، ونسبة نطاق الحدود 10.3٪. وأوصت الدراسة أن اختبار القلوبيين المناعي ج للعدوى لفيروس المضخم للخلايا يجب أن يتم مع كل مقاييس القلوبيين المناعي ج أثناء الحمل.
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CHAPTER ONE
INTRODUCTION

1. Introduction:

Cytomegalovirus (CMV) is a DNA virus, and humans are its only known host. Like other members of the herpes virus family, CMV can cause latent infection. CMV may be transmitted by organ donation, blood transfusion, sexual contact, and contact with infected saliva and urine. At least 50% of women at reproductive age have evidence of prior CMV infection. The prevalence of prior infection is increased in women of lower socioeconomic status. Most pregnant women acquire infection as a result of contact with their own younger children or children in a daycare or pre-school setting (Patrick 2010). Infants may acquire CMV infection by contact with contaminated blood and genital secretions during delivery and via breast milk after delivery. Fortunately, these two mechanisms of transmission are extremely unlikely to cause any injury to the baby. However, when a pregnant woman develops primary CMV infection in the first half of pregnancy, the risk of transplacental infection is approximately 40%, 5 to 15% of these babies, are acutely symptomatic at birth. The principal clinical manifestations of severe congenital CMV infection include hepatosplenomegaly, thrombocytopenia with resultant petechiae, intracranial calcifications, intrauterine growth restriction, hepatitis and jaundice, microcephaly, chorioretinitis, hearing loss, mental retardation, and seizures. Approximately 30% of severe infected infants die and 80% of the survivors have serious sequelae (Patrick 2010). 85 to 95% of infected infants are asymptomatic at birth. 10 to 15% of the initially asymptomatic children subsequently develop neurologic auditory visual, and/or dental defects, which become evident as they enter school. Interestingly, when primary maternal infection occurs in the third trimester of pregnancy, the risk of transplacental transmission is much higher – 75 to 80%. However, the risk of serious fetal injury is very low. In contrast to primary maternal infection, recurrent or reactivated maternal infection does not pose serious risk to the fetus. When recurrent or reactivated CMV infection develops during pregnancy, only 5 to 10% of infants become infected. None of these children are symptomatic at birth. Late sequel of infection includes minor visual and auditory deficits and developmental delays which become apparent as the child enters elementary school (Patrick 2010). The risk of intrauterine transmission of
cytomegalovirus (CMV) during pregnancy is much greater for women who contract primary CMV infection after conception than for women with evidence of infection (circulating CMV antibodies) before conception. Laboratory investigations that used for identification of recent primary CMV infection are important tools for managing the care of pregnant women suspected of been exposed to CMV. CMV IgM detection is a sensitive marker of primary CMV infection, but its specificity is poor because is also produced during viral reactivation and persists following primary infection in some individuals. Studies conducted over the last 20 years convincingly demonstrate that measurement of CMV IgG avidity is both a sensitive and a specific method for identifying pregnant women with recent primary CMV infection and those at increased risk for vertical CMV transmission. IgG avidity is defined as the strength with which IgG binds to antigenic epitopes expressed by a given protein; it matures gradually during the 6 months following primary infection. Low CMV IgG avidity is an accurate indicator of primary infection within the preceding 3 to 4 months, whereas high avidity excludes primary infection within the preceding 3 months (Prince and Leber 2002). CMV causes cytomegalic inclusion disease, especially congenital abnormalities, in neonates. It is the most common cause of congenital abnormalities. CMV is very important cause of pneumonia and other disease in immunocompromised patients. It also causes heterophil negative mononucleosis in immunocompetent individual (Levinson 2013). As with most other herpes-type viruses, once you are infected with CMV, it will remain inactive in your body for the rest of your life. CMV causes few symptoms in most people. It spreads through bodily fluids, such as saliva, urine, and can be passed on through close bodily contact. The infection can be found in small droplets of saliva which are spread from one person to another, when an infected person coughs or sneezes. There is no cure for CMV. Possible vaccines for the condition are currently being researched and used in clinical trials, but a vaccine will probably not become publicly available for several years. CMV infection can be primary due to infection in the first time, or can be reoccurring when the infection is reactivated due to the immune system is weakened, can be reinfection when the infection happen by other strain of the virus and can be congenital CMV when the infection develops in pregnant woman and infects the unborn baby (Prince and Leber 2002). CMV is only serious when it develops or reoccurs in certain groups of people. The two main groups of people at risk
from CMV are people, those with a weakened immune system, particularly HIV infected individuals or who have recently had an organ transplant, bone marrow transplant and unborn babies, if a woman becomes infected with CMV for the first time during pregnancy, there is a risk that she may pass the infection on to her unborn baby. Infection in the unborn baby is known as congenital CMV. It is estimated that 1 to 4 babies in every 200 will be born with congenital CMV. Only 1 in 10 babies who are born with congenital CMV have symptoms at birth. However, these symptoms can be serious and include long-term problems, such as learning difficulties and hearing loss. A few babies who are born with congenital CMV but have no symptoms at birth will experience hearing loss that develops over their first few years of life (Prince and Leber 2002). Infection of the fetus occurs mainly when the mother has primary infection occurs in the beginning of pregnancy, when she has no antibodies that will neutralize the virus before it can infect the fetus. The fetus usually will not be infected if the pregnant woman has antibodies against the virus. Congenital abnormalities are more common when fetus is infected during the first trimester than later in gestation. Because in first trimester the development of organs occurs and the death of any precursor cells will be due to congenital defect. CMV enters a latent state primarily in monocytes and can be reactivated when cell mediated immunity is decreased. Infection with CMV is the most common cause of nonhereditary sensor neural hearing loss (SNHL) (Levinson 2013).
1.2 Problem identification and Justification:

Over the several decades, the prevalence and importance of CMV human's pathogen become more apparent. CMV causes the most common prenatal viral infection in developed countries. CMV infect nearly 1% of all new-borns 40,000 infants per year, in the United States Approximately 20% of infants infected with CMV during gestation show clinically apparent manifestation of cytomegalic inclusion disease such as microcephaly, seizure, deafness, jaundice and purpura, hepatospleenomegally. Cytomegalic inclusion disease is one of the leading cause of mental retardation. In United States infected infants can continue to excrete CMV, specially in the urine for several years (Levinson 2013).
In addition to congenital and prenatal infection, CMV cause significant morbidity in immunocompromised patients (Levinson 2013).
In Sudan there is no available publish data about the prevalence of CMV among pregnant women. So this study was done to evaluate the IgG avidity of CMV infection among pregnant women.

1.3 Objective:

1.3.1. General Objective:

To evaluate the IgG avidity for CMV infection among pregnant women Wad medani, Outpatient, Clinic, Obstetrics and Gynecology Teaching Hospital, Gezira State.

1.3.2. Specific Objectives:

1-To determine and discriminate between high –avidity, low- avidity and the equivocal range among clinical samples.
2- To co-relate results with different range of age and all trimester stages of pregnant women in the study area.
CHAPTER TWO
LITERATURE REVIEW

2.1 History of Cytomegalovirus:
CMV was first noticed by Riber in 1881, when he saw large protozoan like, cells in the kidney of still born infant, the first report of the visualization of CMV was by electron microscope appeared in 1953, when cytomegalic inclusion cells from an infant pancreas were viewed and particles were observed in both the cytoplasm and the clear hallow around the inclusions. The virus was cultured for the first time in 1956 by Rowe, smith and Weller, all of whom worked independently (Bhatia and Ichhpujani 2008). CMV is structurally and morphologically similar to other herpes viruses but is antigenically different, it has single serotype. Animal CMV strains do not infect human; giant cells are performed, so the name cytomegalo (Levinson 2013). CMV is a member of the beta herpesvirinae subfamily of herpesviridae. The herpes viruses share a characteristic ability to remain latent within the body over long periods, Human CMV is an ubiquitous agent. Acute primary infection in immunocompetant children and adult is self limiting followed by virus latency in CD34 haemopoetic progenitor cell in bone marrow and CD13, CD14 peripheral blood monocytes. Congenital CMV and CMV infection in low birth weight (LBW) neonates (Bhatia and Ichhpujani 2008). The risk factor for maternal acquisition of CMV during pregnancy is frequent and prolonged contact with a child less than three years of age, this occurs among women employed in a childcare center's (Adler 2011).

2.2 Classification:
CMV formerly designated as human herpesvirus 5 (HHV-5) by the international committee on taxonomy of viruses, is member of the family herpesviridae, and it’s classified in the subfamily Betaherpesvirinae. Human CMV (HCMV) is the type species of the genus cytomegalovirus, and its name is derived from the enlargement of the cells infected by the virus (cyto=cell, mega=large). Its classification as herpesvirus is based on its tendency to infect mononuclear cells and lymphocytes and on its molecular phylogenetic relationship to other herpesviruses. Its classification as a Betaherpesvirus is based on its long replication
cycle, cytopathology and restricted host range, which are all characteristic of the betaherpesviruses (Todd and Wills 2006).

2.3 Structure of the virus:

Compared to other human herpesvirus, HCMV is the largest with a genome of 235 kb encoding 165 genes. The virion consists of double stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix (the tegument). These components are enclosed in a lipid bilayer envelope that contains anumber of viral glycoproteins, these include glycoprotein B (gB), gH, gL, gM, gN and gO, mature virions range in diameter from 200 to 300 nanometer (Crough and Khanna 2009).

2.4 Prosperities of CMV:

CMV has the largest genetic content of the human herpesvirus. Its DNA genome (240 kbp) is significantly larger than of herpes simplex virus. Only a few of many proteins encoded by the virus have been characterized. A cell surface glycoprotein, act as Fc receptors that can non specifically bind the Fc portion of immunoglobulins. This may help infected cells evade immune elimination by providing a protective coating of irrelevant host immunoglobulins. The major immediate early promoter enhancer of CMV is one of the strongest known enhancers, due to the concentration of binding sites for cellular transcription factors. It is used experimentally to support high level expression of foreign genes. Many genetically different strains of CMV are circulating in the human population. The strains are sufficiently related antigenically, however so that strain differences are probably not important determinant in human disease (Brooks et al., 2010).

2.5 Replication of CMV:

Its replication is similar to that of Herpes simplex virus (HSV). One unique feature of CMV replication is that some of its immediate early proteins are translated from messenger RNA (mRNA) brought in to the infected cell by the parental virion rather than being translated from mRNAs synthesized in the new infected cell (Levinson 2013). Cytomegalovirus attach to cell surface at the site of the receptor for fibroblast growth factor. After entry into the cell, the virion is uncoated and the genome DNA enters the nucleus. Within the nucleus, the incoming genome DNA changes its configuration from
linear to circular. Early virus mRNA is transcribed by host cell RNA polymerase and then translated into early, non structural proteins in the cytoplasm. The viral DNA polymerase replicates the genome DNA, and late protein synthesis begins. Then the virion assembly occur in the nucleus. The virion obtains its envelope by budding through the nuclear membrane and exits the cell via tubules or vacuoles that communicate with the exterior (Levinson 2013).

### 2.6.1 Transmission:

CMV infection occurs worldwide, and more than 80% of adults have antibodies against this virus. CMV is transmitted by a variety of modes, across the placenta within the birth canal. In young children, it’s most common mode of transmission via saliva. Later can transmitted via sexually transmission; as its present in both semen and cervical secretions (Levinson 2013). HCMV can be transmitted via blood transfusion, solid organ transplantation (SOT), or hematopoietic cell transplantation. Day cares centers are significant source of CMV infection. Children less than three years of age with postnataly acquired CMV infection have been demonstrated to excrete CMV transmission in their urine and saliva for 6 to 42 month. CMV transmission in infant breast fed by seropositive women shedding virus in their breast milk has been reported to be 58% to 69% (Nyholm and Scheiss 2010).

### 2.7 Epidemiology:

HCMV is highly species-specific, with human being the only host. Furthermore CMV has been found in every human population tested. The prevalence of infection is greater in developing countries and among lower socioeconomic groups of developed countries. Overall , the seroprevalance of infection varies between 65% to 90% among middle age adult in USA where primary CMV infection during pregnancy occurs in 2% of women of child bearing age who are of lower socioeconomic background (Nassetta et al., 2009). CMV is a herpes virus is endemic throughout the world. Seroprevalance of CMV has been reported to be highest in South America, Africa and Asia, and found to be lowest in Western Europe and United States. Data referring to the prevelance of anti-CMV antibody among healthy people in Iran is scantly but its incidence may reach 100% due to condensed population and socio-economic status (Jamal et al., 2012).
2.8 Pathogenesis:

Cytomegalovirus is an enveloped double stranded DNA virus. On this envelope, there are molecules called glycoproteins. The glycoprotein’s B can cause a humoral immune response. Proteins in the viral tegument, the proteinaceous layer between the capsid and the envelop, elicit cellular responses to CMV, specifically the abundant molecule phosphoprotein 65 is the molecule targeted by cytotoxic T cells in the cellular response. Although the host has the ability to defend itself against a CMV invasion, CMV has the genes that interfere with the antigen presentation of infected cells, thus limiting the immune response to it, therefore it can remain alive for a long time in the host (Riley 1997).

2.9 Congenital and neonatal infection:

The third type of CMV disease is congenital or partial CMV. This is when a woman who is infected with CMV even if she doesn’t have any symptoms passes it to her child. In congenital CMV, pregnant woman passes the disease through the placenta to the fetus. In prenatal CMV, a mother passes CMV to her child through nursing. Severity range from a simple fever of unknown origin, to enlarged liver and/or spleen, and even motor/mental retardation. The spread of the virus is not associated with food or water. It’s generally spread through close contact with bodily fluids of an infected person. When the host is infected, CMV DNA can be detected with polymerase chain reaction (PCR) in all the different cell lineages and organ systems in the body. Upon initial infection, CMV infects epithelial cells of the salivary gland, resulting in persistent infection and viral shedding (Todd and Wills 2006). Infection of the fetus can cause cytomegalic inclusion disease, characterized by multinucleated giant cells with prominent intranuclear inclusions, many organs are affected, and wide spread congenital abnormalities result (Levinson 2013).

2.10 Immunity:

The innate immune system plays an important role in defence against HCMV and also in priming the adaptive immune response. It’s becoming increasingly apparent that HCMV is subjected to innate sensing by toll –like receptor (TLRs). The stimulation of TLRs by pathogens such as HCMV activates signal transduction pathways, which include the
secretion of inflammatory cytokines that recruit cells of the innate immune system, and the up regulation of co-stimulatory molecules such as CD80 and CD86, which are important for activation of adaptive immunity (Boehme and Compton 2008). The establishment of long lasting immunity in response to a primary HCMV infection, which saves to control subsequent HCMV reactivation in the host, is important for preventing uncontrolled replication and serious HCMV disease. HCMV is a potent immunogen that trigger strong immune response from all arms of the immune system, while the contribution of antibodies for protection and control against HCMV has been debated. Evidence does support role for humoral immunity in the effective immune response against HCMV, predominantly in restricting viral dissemination and in limiting the severity of the disease (Boppana and Britt 1995). The cell mediated immune response is the predominant mechanism by which HCMV replication is controlled, as with the exception of congenital infection, sever HCMV disease occurs almost exclusively in patient with profound cellular immunodeficiency (Crough and Khanna 2009).

2.11 Laboratory Diagnosis:

Laboratory diagnosis of CMV infection depend on can be done by the detecting CMV cytopathology, antigen, DNA in infected tissues, isolating the virus from tissue or secretions and demonstrating seroconversion (Drew 2004). Most useful specimens for CMV isolation are throat washing and urine. The virus is shed in urine for months after clinical features have subsided. CMV can also be isolated from saliva, breast milk, cervical secretions, blood and semen as well as various biopsy materials (Bhatia and Ichhpujani 2008). All specimens should be sent to the laboratory without delay, if delay of more than a few hours, samples should be sent refrigerated or on wet ice. Under any circumstances specimen should not be frozen (Griffiths 2004).

2.12 Previous studies:

A study done in France in the year 1997 by L-Keros, on 40 pregnant women, the results showed that pregnant women with past or secondary CMV infection were > 60%. In contrast, most of them with CMV primary infection, and whose are > 3 months after the last seronegative sample, there is no an avidity indices (AI) < 65% which it means all of
them their results high avidity. So an AI >65% is highly suggestive of a non-primary infection.

A study done in Australia in the year 2005 by S. C. Munro, on 600 pregnant women, all samples were investigated for both IgM and IgG. The majority (56.8%) of women were CMV IgG seropositive, with 5.5% being also CMV IgM positive. In the IgM-positive women 1.2% had a low-avidity IgG, indicating a primary CMV infection and a high risk of intrauterine transmission. Two infants with asymptomatic CMV infection were born of mothers who had seroconverted in the second trimester of pregnancy.

A study done in Egypt in the year 2013 by N. Kamel. CMV IgG avidity test performed on samples from 40 women who were positive for both CMV IgG and CMV IgM tests containing also the grey zone(equivocal range). All the 40 samples showed a high and intermediate CMV IgG avidity index. 23% out of them were in the second or third trimesters and had their first-trimester blood retrieved and tested which showed the same results.

A study done in Sudan in the year 2016 by Altayeb, the CMV avidity test was done for 90 pregnant women, they found low avidity CMV IgG antibodies in 1.1% case while 98.9% cases showed high avidity CMV IgG antibodies. Women had high avidity index indicating recurrent or past CMV infection. The age of the study group ranged from 14–24 years, and there was no significant association between CMV infection and age or trimester stages. In addition to the IgG avidity is very important for differentiated between recent or past infection.

A study done in Nigeria in the year 2016 by Abdullahi Nasir. He found functional binding affinity of anti-CMV IgG antibodies increases progressively over time after immunity by infection it is referred to as maturation of the humoral immune response. Low IgG antibodies avidity indices indicate primary infection whereas high avidity indices indicate non-primary infection. In several previous evaluations, (AI) above 60% during the first trimester of pregnancy could reasonably be considered a good indicator of past CMV infection, whereas in women with low, AI less than or equal to 50% indicates there was a risk of congenital CMV transmission.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Design:

Descriptive cross sectional study to evaluate the IgG avidity of human cytomegalovirus among the pregnant women in Wad Medani, Out Patient Clinic Obstetrics and Gynecology Teaching Hospital, Gezira State.

3.2 Study Area:

Study was conducted in Wad Medani Out Patient Clinic, Obstetrics and Gynecology Teaching Hospital, Gezira State.

3.3 Study population:

Study was done on pregnant women whom attended Wad Medani Out Patient Clinic, Obstetrics and Gynecology Teaching Hospital, Gezira State.

3.4 Study Duration:

Study was conducted in duration from October 2017 to December 2017.

3.5 Sample size:

A total of 46 specimens (n=46) were collected from pregnant women and investigated for CMV – specific immunoglobulin IgG avidity.

3.6 The criteria of study:

3.6.1 Inclusion criteria:

All pregnant women attended to Wad Medani Out Patient Clinic, Obstetrics and Gynecology Teaching Hospital, Gezira State, during the study duration with no restriction to age and trimester stages, and willing to participate in the study were included.

3.6.2 Exclusion criteria:

Non pregnant women are excluded.

3.7 Study variable:
3.7.1 Dependant variable:

The study was conducted on IgG avidity.

3.7.2 Independent variables:

Age
Residence
Trimester stage
Occupation

3.8 Data collection:

Personal and clinical data was collected by direct interviewing questionnaire from each subject.

3.9 Data analysis:

The statistical package of social science (SPSS) software program was used for statistical analysis. Significance of difference was determined using chi-square test. Statistical significance was set at <0.05.

3.10 Ethical Approval:

Approval was taken from the Ministry of Health Gezira State, and also had permission from the head director of the Obstetrics and Gynecology Hospital in Wad Medani in Gezira State.

All patients examine was informed for the purpose of the study before collection of the sample and verbal consent was taken from them.

3.11 Method:

3.11.1 Sample collection:

The study based on non-probability convenience sampling technique during attendance of pregnant women to the Obstetrics and Gynecology Teaching Hospital, Wad Medani, Gezira State. The collection of blood samples was done under aseptic conditions, blood samples was allowed to clot and centrifuged at 3000 rpm for minutes. Sera was collect in
sterile container and store at temperature 4°C until test will be performed. The research was done using one ELISA Kits Euroimmun.

3.11.2 Principle:

The qualitative immune-enzymatic determination of specific antibodies is based on the Enzyme Linked Immunosorbent Assay (ELISA) technique. Micro plates, which were coated with specific antigens to bind cross bonding antibodies of the sample (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with washing buffer. The avidity reagent removes the low–avidity antibodies from the antigens whereas the high avidity ones will still bound to the specific antigens. After the second washing step, to remove the rest of avidity reagent and low avidity antibodies, horseradish peroxidase (HRP) labelled conjugate was added, this conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. The immunocomplex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of the product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. A yellow endpoint colour was read at 450/620 nm absorbance using an ELISA micro well plate reader.

3.11.3 Procedure:

Incubation:

- 100µl control or diluted patient samples was transferred into the individual micro-plate wells according to the pepetting protocol and incubated for 30 minutes at room temperature.
- Washing:
- Manual: The wells was emptied, washed using 300 of w µl working strength wash buffer.
- Automatic: Reagent wells was washed with 450 µl of working strength wash buffer using TECAN Columbus Washer Overflow Modus.
- Washing buffer was left in each well for 30 to 60 seconds per washing cycle, and then the wells was emptied. After washing manual and automated tests, all liquid was thoroughly disposed from the microplate by tapping it on absorbent paper with
the openings facing downwards to remove all residual wash buffers. Free positions on the microplate strip was filled with blank wells of the same plate format as that of the parameter to be investigated.

- **Urea incubation:**
- 200 µl of urea solution was added into each of the microplate wells of the first microtiter strip and 200 µl of phosphate buffer into each of the microplate wells of the second microtiter strip, then incubate for 10 minutes at room temperature.

- **Washing:**
- The wells were emptied, washed 3 times using working strength wash buffer for each wash.

- **Conjugate incubation:**
- 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) was added into each of the microplate wells, and incubated for 30 minutes at room temperature.

- **Washing:**
- The wells were emptied, washed 3 times using working strength buffer for each wash.

- **Substrate incubation:**
- 100 µl of chromogen/substrate solution was added into each of the microplate wells.
- Incubate for 15 minutes at room temperature protect from direct sunlight.

- **Stopping the reaction:**
- 100 µl of stop solution was added into each of the microplate wells at the same order and at the same speed as the chromogen/substrate solution introduced.

- **Measurement:**
- Photometric measurement of the colour intensity was done at wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, the microplate was slightly shake to insure homogenous distribution of the solution.

**3.11.4 Limitation and cut-off:**
➢ Controls are ready for use. High control and low control both was treats as patients samples through all test steps.

➢ The presence of low avidity antibodies in a patients serum has been proved in the ELISA extinction value is considerably reduced by urea treatment. In order to objectivate the results arelative avidity index (RAI) is calculated and expressed in percent using the extinction values with and without urea treatment.

\[
\text{Extinction of the sample with urea treatment} \times 100 / \text{Extinction of the sample without urea treatment} = \text{Relative avidity index (RAI) in \%}.
\]

The upper limit of the range of low-avidity antibodies (cut-off value) recommended by (EUROIMMUN is 40% RAI.

Interpretation of the results:
Values below the indicated cut-off are to be considered as an indication of low-avidity antibodies, values between 40% and 60% RAI as equivocal, value above 60% as an indication of high-avidity antibodies. If a result is classified as equivocal, it’s recommended to collect a second sample not less than 7 days later, and to test it together with the first sample.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Results:

The frequency distribution of residence on the study population was taken equal in number from both rural and urban area to make accurate correlation. The urban were 50% and the rural were 50%.

Figure (4.1): Distribution of study population according to Residence.
The age group ranged from 18 to more than 35 which give a wide range of population study to consider variable ages in the study. The frequency of age group 18-26 was 39%, 27-34 was 33 and more than 35 years of age was 28%.

**Figure (4.2):** Distribution of study population according to age.
All trimester stages were included in the study. The frequency of the first trimester was 22%, second trimester was 41% and third trimester was 37%.

Figure (4.3): Distribution of study population according to trimester.
The distribution of the study subjects according to occupation showed that. House wives were 85%, teacher 9%, nurse 4% and students were 2%.

**Figure (4.4):** Distribution of population study according to occupation.
High avidity is (42) 91.3%, 8.7% (4) equivocal range and zero (0) percent for low avidity.

**Figure (4.5):** Distribution of Relative Avidity Index on the study population.
Table (4.1): The distribution of residence according to avidity level.

<table>
<thead>
<tr>
<th></th>
<th>High avidity</th>
<th>Equivocal range</th>
<th>P.value</th>
<th>Odd Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Rural</td>
<td>21</td>
<td>2</td>
<td>0.696</td>
<td>1.0</td>
<td>0.129</td>
</tr>
<tr>
<td>Urban</td>
<td>21</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table shows the same percentage for both rural and urban areas in high avidity and equivocal range respectively. There was no significant association between residence and the result of IgG avidity test for CMV (p.value=0.696).
Table (4.2): The distribution of avidity level according to age group.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>High avidity</th>
<th>Equivocal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 – 26 Years</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>27 – 34 Years</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>&gt;35 Years</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

P=0.126

The table explains that the equivocal range is not found on age group more than 18 and less than 26 years in spite of this group of age contain more number of study populations. And the other age groups their result is near to other.
**Table (4.3):** The distribution of avidity level according to trimester.

<table>
<thead>
<tr>
<th></th>
<th>High avidity</th>
<th>Equivocal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Second trimester</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Third trimester</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>

$P=0.876$

The table shows all trimester stages contain high percentage on high avidity with low percentage on equivocal range.
Table (4.4): The distribution of avidity level according to occupation.

<table>
<thead>
<tr>
<th></th>
<th>High avidity</th>
<th>Equivocal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wife</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>Nurse</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Student</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Teacher</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

P=0.853

In this table the equivocal range is just found on the house wife may be that is due to its high frequency related to other occupations.
4.2 Discussion:

The infection by CMV is the most common cause of congenital malformation resulting from viral intrauterine infection in developed countries. Primary CMV infection can occur during pregnancies and may be transmitted intrauterine to the fetus. CMV infections result in symptomatic congenital disease at birth, and asymptomatic congenital CMV will develop significant clinical sequelae in infancy. In utero transmission of CMV can occur during primary maternal infection, reactivation, or reinfection of seropositive mothers. The investigation of CMV is usually done serologically for both CMV IgG and CMV IgM. Inspite of the detection of CMV IgG avidity is give more reliable result for the recent infection or past infection which it represent a better marker for CMV investigation.

The study was done on 46 pregnant women (n=46), which are distributed on both rural and urban areas (figure 4.1) because there were some studies that correlate between the rural areas and the level of CMV infection which is found high on rural areas related to the urban area. The study population include different range of age group (figure 4.2) (18 to more than 35) years old in which a wide range of age gave us more reliable result for the effect of age on IgG avidity result. All stages of trimester (figure 4.3) were included to ensure and differentiate between trimester stags if there are any effect of them on the result, also the occupation of the study population (figure 4.4) was included in the study as some jobs can enhance the CMV infection eg; health care jobs and nursery. All these are obtained on questionnaire to make specific correlations between all results and then know the effect of every one of these categories on the level of IgG avidity for CMV infection.

All results were illustrated on tables for each category, (figure 4.5) showed the distribution of Relative Avidity Index on the study population, found that high avidity was 91.3% with 8.7% equivocal range and zero percent for low avidity. This result agreed with study done by N. Kamel from Egypt (2013), which all samples showed a high and intermediate CMV IgG avidity index without low IgG avidity test, also agreed with the study done by L-Keros from France (1997), that the high avidity is highly suggestive for non primary infection, and the third study done by S. C. Munro from Australia (2005), which concluded that the majority of women were CMV IgG avidity seropositive, with few incidence of low-avidity IgG, indicating a primary CMV infection.
The distribution of residence according to avidity level (table 4.1), same percentage was for both rural an urban area in high avidity and equivocal range 91.3% and 8.7% respectively. CMV has been found in every human population tested (Nassetta et al., 2009). There was no significant relation between residence and the result of IgG avidity test for CMV in the current study. This result agreed also with Altayeb from Sudan (2016), who found there was no significant association between CMV infection and residence.

According to age group, the distribution of avidity level (table 4.2), the age (18 – 26) years showed 100% high avidity, (27 – 34) years 80% high avidity, 20% equivocal range, and more than 35 years 90% high avidity, 10% equivocal range this result showed significant correlation between age and high IgG avidity specially small age group (18-26) years. The equivocal range was not found in this age group inspite that this group contains more number of study populations. The age group (27-34) years showed 80% high avidity, which is slightly less than the age group (18-26) years that may be the infection by CMV is more common in children, so small age group (18-26) years had high positive results than the age group (27-34) years. Children less than three years of age with postnataly acquired CMV infection have been demonstrated to excrete CMV transmission in their urine and saliva for 6 to 42 month (Nyholm and Scheiss, 2010).

Distribution of avidity level according to trimester (table 4.3), found that all trimester stages are contain high percentage on high avidity with low percentage on equivocal range first trimester 90%high avidity ,10% equivocal range, second trimester 89.4% high avidity,10.6% equivocal range, third trimester 94.1% high avidity, 5.9% equivocal range. This result is agreed with many studies in which there was no relation between trimester stage and the infection by CMV but that is don’t deny the affect of the infection on first trimester and the beginning of second trimester is most harmful for fetus than the infection on third trimester. The fetus usually will not be infected if the pregnant has antibodies against the virus. Congenital abnormalities are more common when fetus is infected during the first trimester than later in gestation (Levinson 2013). This result agreed with Altayeb from Sudan (2016), who found there was no significant association between CMV infection and trimester stages. The study agreed with Abdullahi Nasir (2016) in Nigeria who found that the avidity indices (AI) above 60% during the first trimester of pregnancy it considered
as good indicator of past CMV infection, whereas in women with low AI less than or equal to 50%, there was a risk of congenital CMV transmission.

The distribution of avidity level according to occupation (table 4.4), the equivocal range was found high among the housewives were (89.7%) high avidity may be due to its high frequency related to other occupations and their contact with their babies for long time. Day care centers are significant source of CMV infection (Nyholm and Scheiss 2010).
CHAPTER FIVE
CONCLUSION AND RECOMMENDATION

5.1 Conclusion:
This study concludes that:
1. There was significant correlation between age and CMV IgG avidity test which it found to be 100% high avidity among age group (18 -26) years.
2. There was significant correlation between IgG avidity and occupation (house wives 89.7%).
3. There was no correlation between IgG avidity and residence nor with trimester stags.
4. The IgG avidity is the better marker for detection of CMV infection which is discriminated between past infection and primary infection.

5.2 Recommendation:
1. IgG avidity test for CMV should done with each IgG assays during pregnancy.
2. IgG avidity test for CMV should done for each IgM positive results.
3. Further study with large sample size using monoclonal IgG should be done.
4. Health education is recommended among day care centers and pregnant women to prevent indirect transmission from children to pregnant woman.
5. Urine samples from babies in day care centers should be screened for the presence of CMV.
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Appendix (1)

University of Gezira

Faculty of Medical Laboratory Sciences

Department of Microbiology

Questionnaire about the Evaluation of IgG Avidity in Serodiagnosis of Cytomegalovirus among Pregnant Women in Wadmedani hospital in Gezira State

Personal data:

1) Name: ............................................................................................................................

2) Date of sample collection: ..........................................................................................

3) Telephone number: ......................................................................................................

4) Residence: ......................................................................................................................

5) Age: ..............................................................................................................................

6) Month of gestation: ....................................................................................................... 

7) Numbers of gestations: .................................................................................................

8) Occupation: ..................................................................................................................

9) Laboratory investigation of CMV result: .................................................................